

U 013891-8

Claims:

1. A PCR primer set specific for *Leishmania donovani*, said primer set being (1) a first pair of oligonucleotides having the sequences given by SEQ ID NO.1, and SEQ ID NO.2.
wherein the primer set is effective in a PCR assay for detecting the presence of *Leishmania donovani* infection in samples derived from patients infected by leishmaniasis.

2. A PCR primer set as claimed in claim 1, wherein the primer set is the first pair of oligonucleotides.

3. A PCR primer set as claimed in claim 1 wherein SEQ ID No 1 is 5'-
AAATCGGCTCCCAGGCAGGGAAAC-3'

4. A PCR primer set as claimed in claim 1 wherein SEQ ID No 2 is 5'-
GGTACACTCTATCAGTAGCAC-3'

5. A method of detecting the presence of *Leishmania donovani* in a sample from a patient suspected of leishmaniasis, said method comprising the steps of:
 - a) providing a sample from the patient suspected of being infected with *Leishmania donovani*
 - b) isolating and purifying the nucleic acids from the sample,
 - c) forming a polymerase chain reaction solution containing at least a portion of nucleic acids from step (b), a PCR primer set consisting of SEQ ID Nos. 1 and 2, a mixture of nucleoside triphosphate monomers, and an enzyme *Taq* polymerase in a buffered solution,
 - d) carrying out a polymerase chain reaction on the PCR reaction solution to amplify any *Leishmania donovani*-specific nucleic acid; and
 - e) analysing the *Leishmania donovani*-specific nucleic acids obtained in the polymerase chain reaction using gel-electrophoresis method and staining the resulting gel,

wherein the presence of a band at about 600bp is indicative of the presence of *Leishmania donovani* parasites in the patient.

- 6) A method as claimed in claim 5 wherein the sample is obtained from peripheral blood or skin lesions of the patient.
- 7) A method as claimed in claim 5 wherein the nucleic acids are treated with phenol chloroform and ethanol to isolate purify them.
- 8) A method as claimed in claim 5 wherein the primers are sensitive so as to detect even 10 fg *Leishmania* DNA diluted in 10 million fold excess of human DNA in PCR reactions.
- 9) A method as claimed in claim 5 wherein the PCR reaction is performed in a thermal cycler overlaid with mineral oil.
- 10) A PCR primer set as claimed in claim 1 wherein SEQ ID No 1 is 5'-AAATCGGCTCCGAGGCAGGGAAAC-3'
- 11) A PCR primer set as claimed in claim 1 wherein SEQ ID No 2 is 5'-GGTACACTCTATCAGTAGCAC-3'
- 12) A method as claimed in claim 5 wherein steps of amplifying the *Leishmania donovani*-specific nucleic acid comprises initial denaturation at 94°C for 2 min followed by 40 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min and extension at 72°C for 2 min, and a final extension at 72°C is carried out for 3 min so that multiple copies of the *Leishmania donovani* specific nucleic acid are produced.
- 13) A kit for detecting *Leishmania donovani* in a sample, comprising oligonucleotide primers, wherein the primers comprise SEQ ID No 1 and SEQ ID No 2, and wherein the primers specifically hybridize to the said *Leishmania donovani*.